

DEVELOPMENT OF BIOLUMINESCENCE AND OTHER EFFECTOR  
RESPONSES IN THE PENNATULID COELENTERATE  
*RENILLA KÖLLIKERI*

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In benthic marine invertebrates with planktonic larval forms, the conversion from larva to adult usually involves significant morphological and physiological changes. The morphological changes can involve destruction and resorption as well as elaboration and reorganization of larval structures, the production of new tissues and structures, and renewed differentiation of adult organ rudiments (see, for example, Bonar, 1978; Cameron and Hinegardner, 1974, 1978). Despite a wealth of information concerning morphological changes which occur at metamorphosis of marine invertebrate larvae, little is known about the physiological changes at this critical time. In particular, little is known about functional changes in the nervous and effector systems during larval maturation and transformation to the adult form.

The ideal preparation for studying changes in larval neuroeffector function is one in which the organization of nervous and effector elements is simple, the systems in question can be easily monitored, the adult neuroeffector systems are well understood, and the larval stages are relatively large and easy to raise through metamorphosis. These conditions are approached in the octocoral *Renilla köllikeri*. The nervous elements of *Renilla* are arranged in a diffuse neural network (Satterlie, Anderson and Case, 1976). This nerve net is through-conducting, that is, an impulse initiated in one area of the net is transmitted throughout the remaining portion without decrement (Anderson and Case, 1975; Satterlie, Anderson and Case, 1976). Effector function in *Renilla*, and other anthozoans, is based on perhaps the simplest integrative mechanism, peripheral frequency dependent facilitation (Pantin, 1935; Parker, 1920; Nicol, 1955a, b) and is therefore relatively predictable. Although larvae of *Renilla* are not large enough for electrophysiological recording, they possess an effector system, the bioluminescent system, which is easily monitored photometrically. Furthermore, in live preparations, the luminescent cells (photocytes) fluoresce when illuminated with the proper exciting wavelength of light. The luminescent system can therefore be monitored both morphologically and physiologically in live, intact specimens. The neuroeffector systems of adult *Renilla* colonies, including the luminescent system, have been examined in some detail (Anderson and Case, 1975). Electrical impulses were recorded from a colonial conduction system which controls bioluminescence, polyp withdrawal and colonial contraction. Senni-autonomous polyp conduction systems are also active (Anderson and Case, 1975).

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Our studies indicate that the development of *Renilla köllikeri* is similar to that of *Renilla reniformis* (Wilson, 1883) and other octocorals (Matthews, 1916; Gohar, 1940a, b; Chia and Crawford, 1973). The eggs of *Renilla* are large and yolky, and are usually spawned in large numbers. A swimming planula settles and metamorphoses into a primary polyp. Secondary polyps bud from the primary polyp to form the colony (see Wilson, 1883).

We have followed the development of *Renilla* from the fertilized egg to the mature primary polyp during three reproductive seasons in order to describe the onset of effector form and function, with particular reference to three activities: ciliary swimming, muscular activities, and bioluminescence. The observations of effector function have been utilized to describe the functional development of the nervous system(s) that underlie effector activation during the various stages of larval life. In another report, these observations will be supplemented by an ultrastructural investigation of the developmental stages.

### MATERIALS AND METHODS

Colonies of *Renilla köllikeri* Pfeffer were collected in shallow water off Zuma Beach, California and in the Santa Barbara Channel by divers. Healthy specimens were kept in large aerated aquaria at 18 to 20° C and checked several times daily for spontaneous spawning. Colonies that had been dark conditioned for 1 to 2 days in running seawater aquaria frequently spawned following transfer to lighted battery jars of still sea water. Larvae were reared in 3-liter flasks or beakers of aerated sea water, changed twice daily. Late swimmers (see results for description) were transferred to 250-ml beakers with or without sand. The lecithotrophic eggs permitted development to the primary polyp stage without feeding.

### Fluorescence

Living larvae were observed in one of three types of squash preparations. Light and medium squashes involved bridging a coverslip across two other coverslips (No. 1½ thickness) on a slide. For light squashes, the slide was flooded with sea water and for medium squashes, the animal was placed in a small drop. In heavy squashes, which frequently resulted in tissue damage, a coverslip was placed directly on a small drop of sea water containing the specimen. A Zeiss Universal Research Microscope was used with mercury lamp illumination, BG 12 and BG 38 exciting filters, and a 500 nm cut-off barrier filter. Larvae were anesthetized in a 1:1 solution of 0.37 M MgCl<sub>2</sub>: sea water for photography.

### Bioluminescence

Bioluminescence was detected with an EMI 9601B end-window photomultiplier tube operated at -950 V, giving a high signal-to-noise ratio. A Uniblitz 100-2 electric shutter was threaded onto the window end of the tube and operated from outside a dark experimental box. Signals from the photomultiplier tube were amplified with a Keithley 427 current amplifier leading to a 7P1F DC amplifier of a Grass 79D polygraph.

The larvae were tested in laminated plexiglass blocks with 2.5 cm diameter and

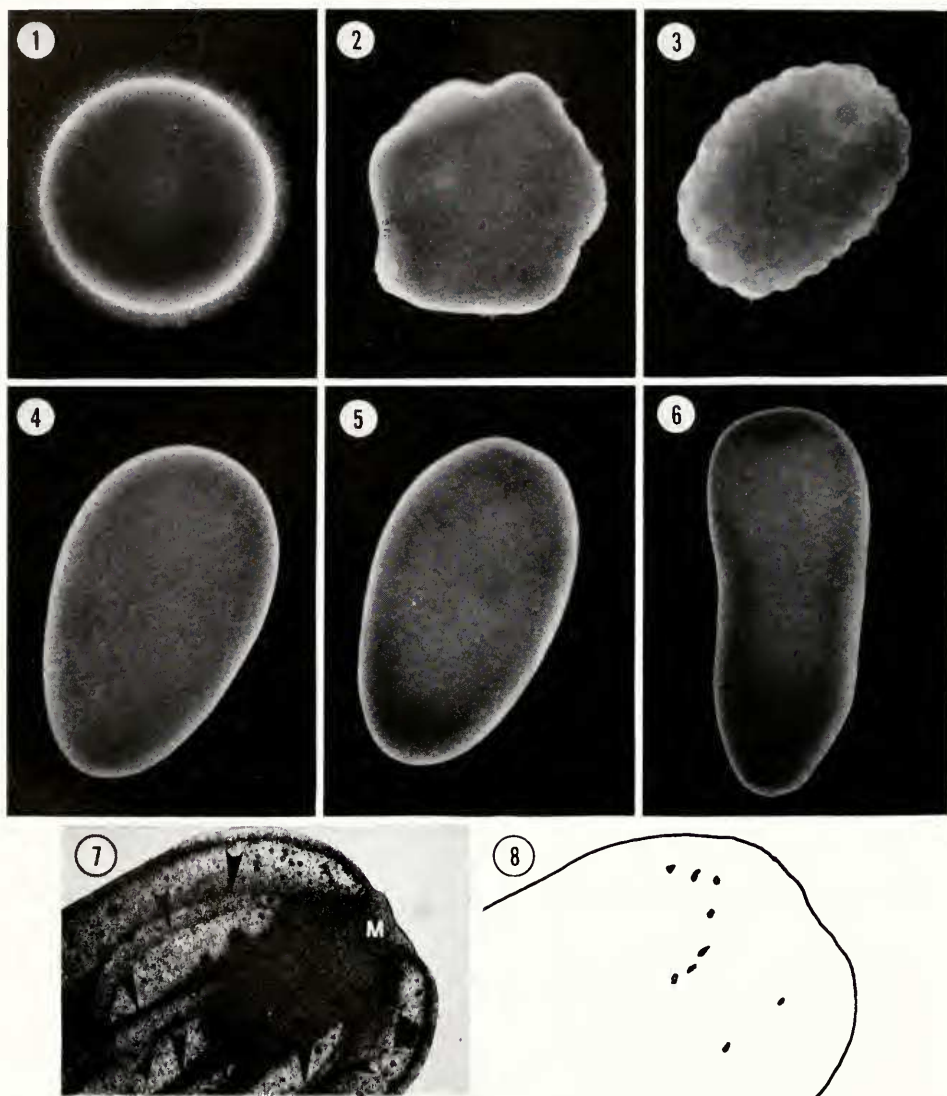


FIGURE 1. Fertilized egg approximately 15 min after release. The ciliary coating is made up of thousands of sperm adhering to the egg surface (verified by E.M.).  $\times 76$ , light squash.

FIGURE 2. About 8-cell stage. The initial cleavages are irregular and apparently incomplete. At this stage, most of the sperm coating is gone. One hour embryo.  $\times 76$ , light squash.

FIGURE 3. About 64-cell stage. Three hour embryo.  $\times 76$ , light squash.

FIGURE 4. Pre-swimmer planula. At this stage, the endoderm is fully formed and the gastric cavity begins to appear. Ciliary activity is weak so the pre-swimmer merely spins in circles on the bottom.  $\times 76$ , light squash.

FIGURE 5. Early swimmer planula. Ciliary activity is sufficient to lift the planula off the bottom. No muscular activity is evident.  $\times 76$ , light squash.

1-cm-deep center wells (same diameter as shutter opening) which were positioned to allow a 3-cm specimen-to-pm tube window distance. Chemicals could be introduced via PE 50 intramedic polyethylene tubing penetrating the wall of the chamber. Single larvae were placed in 1 ml of sea water in the center well, which could be centered under the shutter/pm tube by touch. One milliliter of test solution was slowly injected. The test solutions were 0.53 M KCl, 0.1 M  $\text{CaCl}_2$ , 3%  $\text{H}_2\text{O}_2$ , and sea water controls. The electrical test chamber consisted of a similar plexiglass block with a pair of horizontal 180 mesh silver screens (Unique Wire Weaving Co., Hillside, N. J.), leading to a Grass S9 stimulator. Test animals were placed on the bottom screen with enough sea water to cover them but prevent swimming. The upper screen was then pressed down to contact the larva or sea water. Large stimulating voltages were frequently required due to the shunting effect of the sea water. All luminescence experiments were carried out in a dark box within a darkroom at ambient air temperature ( $20^\circ$  to  $25^\circ$  C).

## RESULTS

Pennatulid colonies have three distinct parts: the peduncle, rachis, and polyps. The basal peduncle is an anchoring device which is inserted into the substratum by peristaltic movements. The rachis, or main tissue mass, supports an array of two types of secondary polyps, the siphonozooids and the autozooids. The rachis and peduncle are derived from the primary polyp, and the colony is formed by budding of the secondary polyps (Wilson, 1883). The rachis of *Renilla köllikeri* is flattened and leaf-shaped. The ventral surface is devoid of polyps and normally lies in the substratum. The dorsal surface bears scattered autozooids, inhalent siphonozooid clusters, and a single exhalent siphonozooid.

*Renilla köllikeri* colonies are dioecious. Gametes are borne on the autozooid septal filaments. When mature, the eggs are tan in color and approximately 0.3 to 0.4 mm in diameter. In male colonies, sperm are packed in follicles which are the same size as the eggs, but white in color. Both eggs and sperm follicles are covered by a single layer of ciliated endodermal cells until spawning takes place. During the reproductive season, the septal filaments of each autozooid contain more than 20 sperm follicles or eggs at different stages of development.

### Spawning

The reproductive season for *Renilla köllikeri* in the Santa Barbara area extends from May to late July or early August, a period centered around the summer solstice. During this period colonies may spawn many times, in small groups or *en masse*. In female colonies, spawning begins with an extreme inflation of the

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FIGURE 6. Swimmer planula. The planula is now hollow and the septa begin to form by outgrowth of the endoderm. Swimming is active. Local muscular contractions can be elicited.  $\times 76$ , light squash.

FIGURES 7-8. Swimmer planula, approximately 10 to 15 hr older than in Figure 6. The same animal was used for both bright field (7) and fluorescence (8, traced from original to show position of dimly fluorescent photocytes) micrographs. The first sign of fluorescence is evident at this time. Note that the anthocodial septa are well formed (Fig. 7, arrows). The mouth (M, Fig. 7) is not yet open.  $\times 56$ , heavy squash.

rachis, in conjunction with accelerated and pronounced rachidial peristalsis. In a 9.5 cm colony, each peristaltic wave passed across the rachis in 45 to 60 sec, a conduction velocity of 0.16 to 0.21 cm/s (21° C). This is compared to a rate of 0.11 to 0.13 cm/s (23° C) in non-spawning *Renilla*. Two and sometimes three waves are present on the rachis of spawning colonies at all times. During spawning, the autozooids remain extended with the tentacles bent slightly in an aboral direction. In female colonies, the shedding of eggs appears to result from the peristaltic movements. In one instance, three waves were required to squeeze an egg out of the autozoid mouth.

When released, the eggs are oblong, but become sperical in 15 to 30 min (Fig. 1). They are neutrally buoyant, and float at all levels in an aquarium of still water. All released eggs, as well as some dissected from a spawning colony, were already fertilized, indicating that fertilization occurs before release. Sperm follicles are not released intact during spawning. The follicles rupture within the autozooids, and the sperm are released through the autozoid mouths and the exhalant siphonozoid.

TABLE I

*Summary of early development of Renilla köllikeri with observations on effector function (25° C). The numbers in parentheses indicate the corresponding text figures.*

Hours from spawning	Stage	Ciliary swimming	Muscle activity	Fluorescence	Bioluminescence
0	Fertilized egg (1)	—	—	—	—
$\frac{1}{2}$ -3	First cleavages (2)	—	—	—	—
1-3	About 64-cell (3)	—	—	—	—
7	Stereoblastula	—	—	—	—
32	Pre-swimmer (4)	Spinning on bottom	—	—	—
38	Early swimmer (5)	Swimming	—	—	—
60	Swimmer (6)	Swimming	Local contractions	—	—
84	Swimmer (7)	Swimming	Conducted contractions	First sign (8)	—
118 $\frac{1}{2}$	Late swimmer (9)	Swimming, sinking	Conducted contractions	++ (10)	—
131	Settled (12)	Ceases	Conducted contractions	+++ (11)	— (20B)
136 $\frac{1}{2}$	Settled (13)	—	Separate responses	+++ (13) as in adult	First Sign (20C)
191	Tentacle buds (14)	—	Separate responses	+++	++ (20E)
274	Pinnules (15)	—	Separate responses	+++ (15, 16)	++ (20F)
323	Primary polyp	—	Separate responses	+++ (17)	+++ (20G)



*Larval development*

The early cleavages and larval development of *Renilla köllikeri* are similar to that described by Wilson (1883) for *Renilla reniformis*. The initial cleavages are extremely irregular and variable. Seldom can 2-, 4-, or 8-cell stages be recognized. The first detectable cleavages (*Renilla köllikeri*) occur 20 min to 3 hr after spawning, at which time embryos of 8 to approximately 64 cells are observed (Fig. 3). Prior to this stage the embryos are very irregular in shape (Fig. 2).

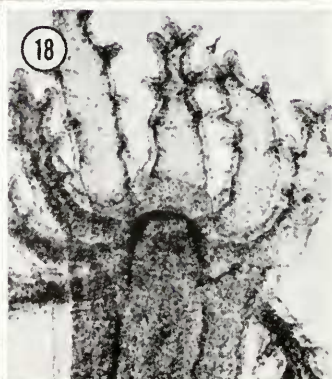
Early development progressed more rapidly at 25° C than at lower temperatures tested. Cooler temperatures, down to 6° C, retarded development without apparent harmful effects. A timetable for the development of *Renilla köllikeri* (25° C) is shown in Table I, and all further mention of age will refer to this table and temperature.

Just prior to cleavage, neutral buoyancy is lost, and the embryos settle to the bottom of the aquarium. No ciliary activity is apparent. Subsequent cell divisions give rise to stereoblastulae. Endoderm formation is followed by gradual disappearance of the central, yolky cells. Swimming planulae are formed 38 hr after spawning (Figs. 4, 5). The planulae are barrel-shaped and swim at the air-water inter-



FIGURE 9. Late swimmer planula. The eight anthocodial septa (one indicated, large arrow) are visible, two of which extend to form the peduncular septum (small arrow).  $\times 101$ , light squash.

FIGURE 10. Late swimmer planula. Tracing of a fluorescence micrograph indicating the position of the dimly fluorescent photocytes, which are arranged in rows along the septa.  $\times 47$ , medium squash.



face (early swimmers, Table I). During the swimming period (4 to 6 days) the planulae gradually elongate (swimmers, Figs. 6, 7) and undergo considerable cellular differentiation. During this time the peduncular septum, as well as the eight anthocodial septa are formed (Fig. 7). The differentiation of muscle and nerve cells begins early in the planula stage (early swimmers; Satterlie and Case, in preparation).

When 5 days old, the planulae (late swimmers, Fig. 9) begin to sink to the bottom of the aquarium. The larvae settle oral end first on clean glass or in sand (Fig. 12). Settlement is achieved more rapidly when a sand substratum is provided, as opposed to clean glass, the difference being as much as 8 hr. Once attached to the substratum by the oral end, the larvae "roll" to the side and eventually attach by the aboral peduncle. The oral end is then raised. When settled in sand, the larvae move between sand grains and in some cases burrow in approximately a half-centimeter.

Tentacle buds first appear as conical projections on the anthocodium at 8 days (Fig. 14). The tentacles gradually elongate and sprout lateral pinnules (Fig. 15). At 13 days, the juveniles represent structurally complete primary polyps (Figs. 18, 19). The juveniles survived as single polyps for up to 3 weeks in the laboratory, but did not bud secondary autozooids. See Wilson (1883) for an account of colony formation in *Renilla reniformis*.

#### *Development of effector systems*

*Ciliary swimming.* The ectoderm of adult specimens of *Renilla* is not heavily ciliated except for the invaginated pharynx of each polyp. However, for a short time during development, the planulae rely on a dense ectodermal ciliary coat for locomotion. Ciliary activity is continuous, and no ciliary reversals or arrests could be demonstrated. When a planula encountered a solid object, for instance the side of a container, it would continue to swim in a forward direction (aboral end first) until it eventually slid away. The swimming motion produces a rotation about the oral-aboral axis, and in the late swimmer, which maintains a bend in the aboral end, produces a cork-screw-like motion. All ectodermal ciliary activity ceases upon settlement. If larvae are immediately dislodged, however, ciliary activity reappears. This ability is not apparent after the larvae have remained

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FIGURE 11. Freshly settled planula. Fluorescence micrograph showing the photocytes arranged along the septa.  $\times 56$ , heavy squash.

FIGURE 12. Freshly settled planula, longitudinally contracted.  $\times 78$ , light squash.

FIGURE 13. Settled juvenile. This animal produced a luminescent response similar to that in Figure 20C. The fluorescent cells are as bright as those in the later stages. Note the constriction in the "neck" region. The anthocodium is inflated, and the peduncle deflated, demonstrating the separation in responses in the two areas.  $\times 58$ , medium squash.

FIGURE 14. Tentacle bud stage. Again note the inflated anthocodium and contracted peduncle.  $\times 80$ , light squash.

FIGURE 15. Tentacled polyp at stage of pinnule development. Note the photocyte processes.  $\times 66$ , medium squash.

FIGURE 16. Higher magnification fluorescence micrograph of the photocyte cluster marked by the arrow in Figure 15.  $\times 214$ , medium squash.

FIGURES 17-18. Anthocodial region of a primary polyp. The photocytes are found in the lateral sides of the tentacle bases.  $\times 43$ , medium squash.





FIGURE 19. Full view of a primary polyp. Two siphonozooids are visible (arrows).  $\times 14$ , medium squash.

settled for an hour or more. If the larvae are not allowed to settle, the late swimmer stage is prolonged and further morphological changes, such as tentacle budding, are delayed.

*Muscular activity.* Obvious muscular activity is lacking until the swimmer stage (60 hr), after elongation is underway. At this point a mechanical or electrical stimulus to any part of the planula produces a local contraction which pulls the stimulated tissue away from the probe. This muscular contraction is not conducted circularly or longitudinally. Within 2 to 5 hr, similar stimuli produce a more widespread circular constriction, and the planulae contract longitudinally to about two thirds of the relaxed length. At this time, the septa are well formed (Fig. 7). By the late swimmer stage, the larvae are capable of bending movements as well as "protective" longitudinal contractions. These conducted muscular events are not observed when the planulae are placed in excess  $Mg^{++}$  for 5 min. In the anesthetized state, stimulation only produces local twitches as in the earlier planulae.

At the time of planula attachment, a division between polyp and peduncle reactions is evident. A stimulus to the peduncle produces a conducted contraction, but not always with an accompanying polyp contraction. Similarly, polyp stimulation does not always produce peduncular contractions. As soon as tentacle buds

are apparent, the primary polyp behaves much like the autozooids of a mature colony. An electrical or mechanical stimulus to the polyp causes an inversion of the anthocodium and an overall contraction of the polyp.

Peristaltic movements are not apparent until settlement, when the peduncle begins rapid, strong peristaltic contractions. If an animal is dislodged, the peristaltic contractions cease until resettlement occurs. As with muscular reactions, a definite separation is evident between the anthocodium and the peduncle, and each is capable of separate peristaltic and bending movements.

### *Development of the bioluminescent system*

**Fluorescence.** The first sign of fluorescence occurs in the swimmer stage approximately 80 hr after spawning. Extremely dim fluorescent cells are evident on the eight anthocodial septa (Figs. 7, 8). The cells are oval to round, 5 to 10  $\mu$ m in maximum length, and without noticeable processes. The size of the fluorescent cell bodies does not appear to change much as the larvae grow. Cell processes are first observed as small, stubby projections after settlement has taken place. By the time of tentacle pinnule development, the cells appear morphologically similar to photocytes of mature autozooids (Figs. 15, 16).

The intensity of fluorescence increases until around 140 hr, when it is approximately as intense as in mature autozooid photocytes. No subsequent increase in fluorescence intensity is apparent. If larvae are prevented from settling, fluorescence increases in intensity at the normal rate, but the appearance of the photocyte cytoplasmic processes is delayed. The increase in fluorescence is not affected by the type of settling substratum.

TABLE II

*Summary of chemical bioluminescence tests expressed as number of successful trials/number of trials. The data on substrate effects are from a separate experiment using KCl as the stimulant.*

Larval stage	KCl	CaCl <sub>2</sub>	H <sub>2</sub> O <sub>2</sub>
64 Cell stage	0/30	—	0/5
Early swimmer	0/33	—	0/5
Swimmer	0/26	0/1	0/5
Late swimmer	0/34	0/2	0/5
Settled (oral attachment)	1/24	0/2	0/5
Settled (peduncle)	13/16	1/2	3/5
Swimmers (same age as peduncle settled)	0/14	—	—
Tentacle buds	23/23	2/2	4/5
Swimmers (same age as tentacle buds)	8/12	—	—
Mature tentacles	11/11	5/5	5/5

Substratum effect on KCl response—peduncle settled larvae approximately 160 hr from spawn

Glass settled (no sand)	5/32		
Sand settled	19/20		
Glass settled (sand in container)	5/8		

As the tentacles begin to form, the fluorescent cell groups appear more peripherally in the septa (compare Figs. 8, 10, 11, 13, 15, and 17). When the tentacles are fully formed, the fluorescent cells are found between the tentacle bases (Fig. 17) as in mature autozooids.

**Bioluminescence.** The bioluminescent capability of *Renilla* was tested with three chemicals known to induce luminescence in adult animals. Potassium chloride, isotonic with sea water, produces transient flashing presumably by randomly depolarizing cells.  $H_2O_2$  produces similar results. Hypotonic  $CaCl_2$  was used by Anderson and Cormier (1973) to induce luminescence from lumisomes, isolated subcellular particles which contain all components of the luminescent reaction. In severed autozooids from adult colonies, 0.1 M  $CaCl_2$  produces steady glowing for up to 45 min.

No bioluminescence was measurable up to the time of planula settlement (Table II and Fig. 13). Even when 12 late swimmer larvae, ready to settle, were stimulated together with KCl, luminescence was still not recordable. For comparison, *Obelia* sp. hydroids were tested with KCl. In *Obelia* the photocytes, which are fluorescent, are scattered in the stolons and uprights. Thus a piece of tissue can be dissected which contains only one photocyte. The record from a test of such a piece of *Obelia* is shown in Figure 20A, and represents a base of comparison for the *Renilla* luminescence tests.

Freshly settled planulae do not luminesce (Fig. 20B). One to three hours after settlement, the first light is recorded (Fig. 20C). At this stage, the juveniles are attached by the peduncle with the anthocodium raised, and the fluorescence intensity of the photocytes is equal to that of mature autozooid photocytes. In general, no bioluminescence is recorded from animals in which the fluorescence is less intense than in adult autozooids. If larvae are detached prior to rolling over to the peduncular attachment, and prevented from settling, luminescent competence is delayed by up to 24 hr (Table II).

The substratum is important to the development of luminescent ability (Table II). Late swimmers will settle on the sides and bottom of glass beakers if no other substratum is available. Appearance of bioluminescence in glass settled juveniles is delayed just as if settlement is prevented. Occasionally, planulae settled on the glass sides of a sand-substratum container, in which case luminescence developed normally.

The waveform of chemically-induced luminescence is initially irregular (Figs. 20C, D). When approximately 190 hr old, the juveniles exhibit a luminescent waveform similar to that of the adult, namely a rapid rise to peak followed by a slower decay (Figs. 20E-G). At the tentacle bud stage, light emission takes the form of several individual peaks (Fig. 20E) possibly indicating the sequential stimulation of photocytes or photocyte groups. By the tentacled stage, the emission consists of broad flashes up to 4 sec in duration (Fig. 20G). Multiple flashes are frequently encountered, with the initial flash normally being the brightest (Fig. 20F). In general, the intensity of bioluminescence increases with age of the juvenile. The above descriptions of the stages of bioluminescent competence represent the norm of multiple tests. The developmental rates of individuals vary slightly.

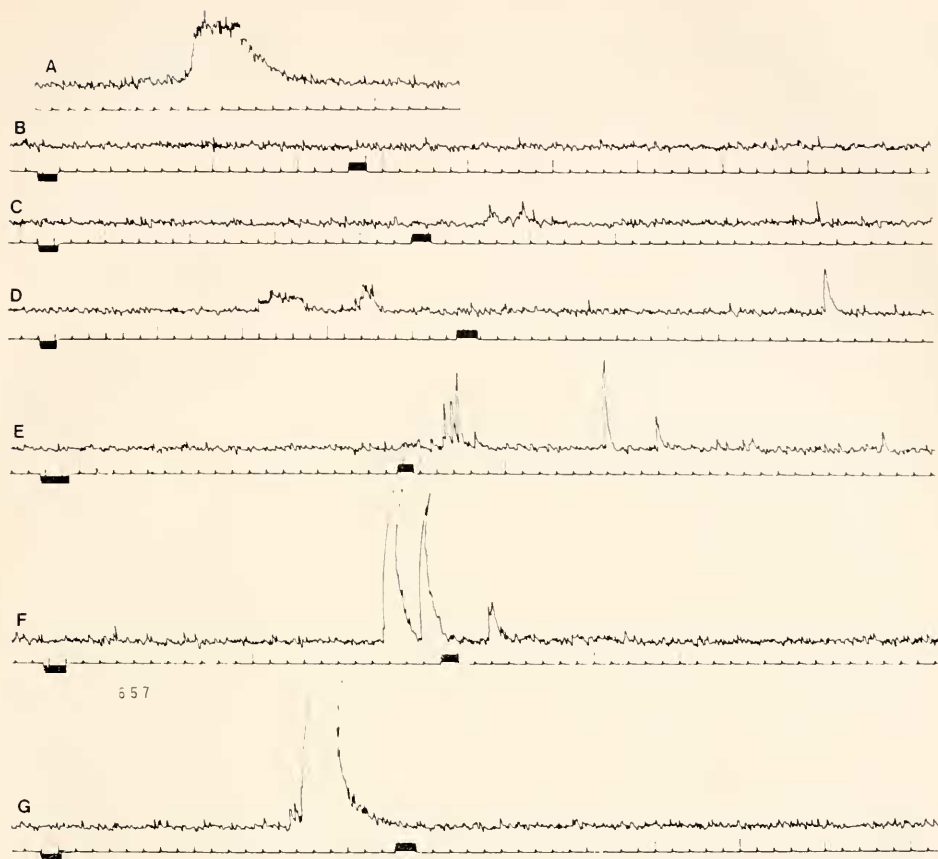


FIGURE 20. Chemically induced bioluminescence in *Renilla* larvae (B-G, see Table II). (A)—KCl induced bioluminescence from a piece of *Obelia* sp. upright containing only one photocyte. The gain is the same for all records. An event marker, superimposed on the time trace (1-second intervals), was used to indicate the beginning (downward mark) and end (upward mark) of the KCl injection. (B)—Newly settled planula; (C)—Settled planula, attached by peduncle; (D)—Juvenile settled for 12 hr; (E)—Tentacle-bud stage; (F)—Pinnule development stage; (G)—Primary polyp. Bioluminescence could not be induced prior to planula settlement.

Settled juveniles luminesce in response to electrical stimulation. Up to the tentacle bud stage (Figs. 21A, B), the voltages required (60 to 80 V/5 ms) result in tissue damage. The responses, however, are similar to those of the KCl tests (Figs. 21A ~ 20C, D; 21B, C ~ 20E; 21D ~ 20F; 21E, F ~ 20G). At the tentacle bud stage and later, the luminescent flashes occur in a 1:1 ratio with stimuli, although in some cases several stimuli are required to initiate the response (Fig. 21C). In subsequent stages, the responses begin with the first or second stimulus (Figs. 21D, E). Stimulus thresholds are on the order of 20 to 40 V/5 ms. Facilitating responses (Fig. 21F), characteristic of adult specimens of *Renilla*, are obtained when the tentacles are fully formed, 320 to 370 hr after spawning

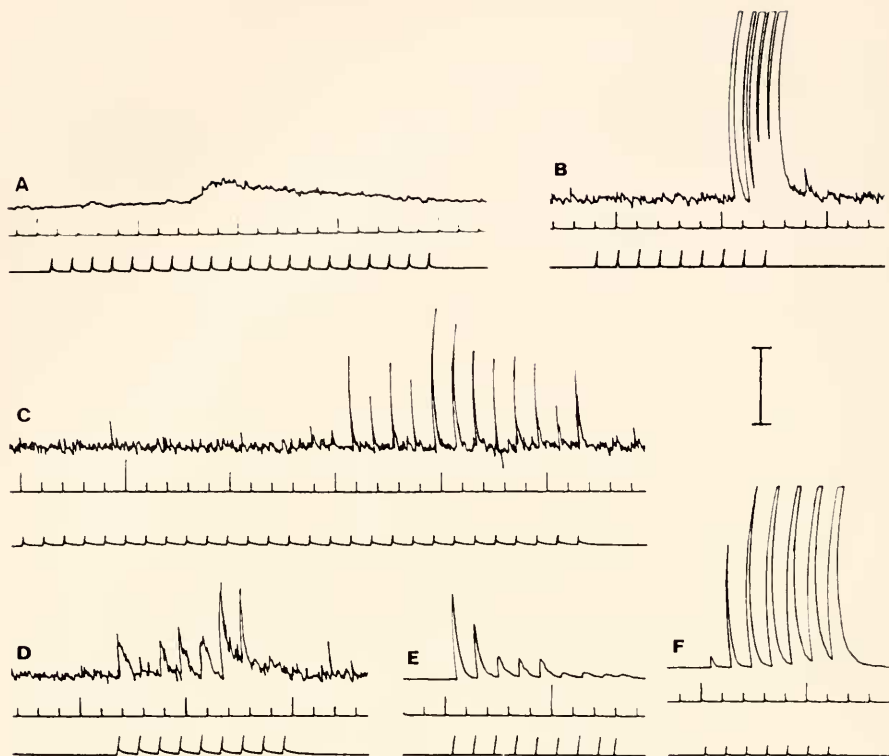


FIGURE 21. Electrically induced bioluminescence in *Renilla* larvae. The top trace represents light output; the center trace, time (1-second intervals); and the bottom trace, stimulus markers (1/s). The scale bar represents the amplitude of the single *Obelia* photocyte luminescent response (Fig. 20A). The gain for traces (A–D) are the same as in Figure 20. The gain for (E) is reduced by 2.5 times, and that for (F) by 5 times. (A)—Settled planula; (B, C)—Tentacle bud stage; (D)—Pinnule development stage; (E, F)—Primary polyp. Bioluminescence responses are initially irregular, eventually following stimuli 1:1. The final stage of maturation of the bioluminescent system is the appearance of facilitating responses (F) similar to those of adult colonies.

(Figs. 18, 19). The stimulus threshold for bioluminescent responses of the mature primary polyp is as low as 5 V/1 ms.

### DISCUSSION

The initiating stimuli for spawning in *Renilla köllikeri* are unknown although light seems to be important. Wilson (1883) found that *Renilla reniformis* spawned between 5 AM and 7 AM in the laboratory. Although *Renilla köllikeri* spawned at all times of the day, most spontaneous spawning occurred between 12 noon and 3 PM. By altering the light regime, small spawns could be induced. Released sperm are swept into the female colonies and circulated by the water vascular system, into which the ripe gonads extend. Fertilization is probably internal and perhaps



stimulates female spawning. This behavior could serve to increase the probability of egg fertilization, which would otherwise be jeopardized by the surging currents in which *Renilla* colonies are found (Kastendiek, 1976).

Dispersal of *Renilla* larvae has two phases. The neutral buoyancy of the fertilized eggs allows them to be swept away from the parent colonies. Following loss of neutral buoyancy in the early cleavage stages, the swimming planulae re-enter the water column for 4 to 6 days.

The ciliary swimming behavior of *Renilla* planulae is noteworthy in that a coordinated ciliary reversal or arrest response is lacking. The forward propulsive beat continues regardless of obstacles. A reversal or arrest would require some form of cell-to-cell communication, be it mechanical or bioelectrical. In larvae of many organisms (see Spencer, 1974), as well as in many adult coelenterates (Mackie, 1965, 1975, 1976; Mackie and Passano, 1968; Spencer, 1971, 1975, 1978) impulses are conducted in excitable epithelia. Such a conduction system seems not to be involved in the swimming behavior of *Renilla* planulae unless in the normal swimming metachronism. Intercellular gap junctions could not be found in any of the larval stages of *Renilla* (Satterlie and Case, in preparation).

Muscular activity is first apparent in the swimmer stage, mostly as local responses. The coordinated contractions of late swimmers suggest the presence of a functional conduction system. Both nervous and muscular elements are present by the swimmer stage, and may represent components of this conduction system (Satterlie and Case, in preparation). It is also possible, however, that coordination is due to electrically or mechanically coupled muscle or epithelial cells. Mechanical coupling is counterindicated by the magnesium sensitivity of the muscular responses, which suggests neural control of musculature at this early stage. At least one neural conduction system is apparently present and functional prior to settlement and metamorphosis.

A proper substratum promotes planula settlement. Chia and Crawford (1973) found that sand grain size was not important to the settlement of the pennatulid *Ptilosarcus gurneyi*, but that an organic film on the sand grains was essential to induce settlement. Müller (1973) found that in the hydrozoan *Hydractinia* settlement and metamorphosis is triggered by a lipid produced by marine bacteria. Our findings suggest that a similar situation exists in *Renilla* since settlement of planulae is delayed if clean glass is the only available substratum. As in other octocorals, prevention of attachment prevents or delays metamorphosis. In several xeniid and gorgonian octocorals, delayed settlement increased the number of abnormalities, such as retarded tentacle development (Gohar, 1940b) and axial skeleton formation (von Koch, cited in Gohar, 1940b). Chia and Crawford (1973, 1977) found that planulae of *Ptilosarcus* would continue to swim indefinitely if the proper substratum was not available. Comparing planulae in which settlement was prevented to primary polyps of the same age, they noted significant differences in ultrastructure despite the identical ages of the two groups. Of 9 cell types in the planulae, and 12 cell types in the polyps, only 7 were common (Chia and Crawford, 1977). This reorganization in cellular composition can be attributed to settlement-metamorphosis. Similar settlement-induced changes may parallel the physiological changes which are apparent in the three effector systems

of *Renilla* at the time of peduncle attachment and metamorphosis. The first of these changes is that the ectodermal ciliation becomes inactive and the cilia are apparently resorbed. A second change is that peristaltic movements of the peduncle become intense and a separation can be seen between the muscular activities of peduncle and anthocodium. A separation of conduction systems, into peduncular (future colonial) and anthocodial (future polyp), is therefore suggested. This separation was shown electrophysiologically in adult *Renilla* by Anderson and Case (1975). The metamorphosis-induced changes in neuromuscular organization of *Renilla* larvae probably involve regional differentiation of the planula conduction system since the reactions of the anthocodium and peduncle do not change. Stimuli to either area still produce conducted muscular activity in the stimulated region. During the third change, the juveniles gain the ability to bioluminesce (when about 5½ days old). The green fluorescent protein (see Morin, 1974; Cormier, Hori and Anderson, 1974; Cormier, Lee and Wampler, 1975 for reviews of *Renilla* bioluminescence biochemistry) is produced prior to settlement, and its manufacture is not drastically altered during metamorphosis. The photocytes of the primary polyp are already differentiating prior to settlement, although some morphological changes do occur at this time, such as growth of photocyte processes. The cause of the immediate appearance of bioluminescent competence is not known. The possibility exists that the bioluminescent system was functional prior to settlement, and we were unable to detect the light. However, the multiple animal tests tend to refute this claim. Also, it would be difficult to explain why delays in settlement would also delay the onset of measurable light output.

Colonial bioluminescent responses in adult *Renilla* colonies are controlled by a colonial nerve net (Anderson and Case, 1975; Satterlie, Anderson and Case, 1976). Light emission generally occurs in a 1:1 ratio with stimuli, after the first, and exhibits a facilitation in intensity which is dependent upon interpulse interval (Nicol, 1955a, b). The primary polyps do not exhibit similar responses until tentacle maturation. The sequence of luminescent responses represented in Figures 21C-F could reflect maturation of the neuro-effector hook-ups (within the polyp system), of the conduction systems (colonial and polyp), of the connection between the two conduction systems, or a combination of such developments. Maturation of the polyp neuro-effector system appears to occur between the tentacle bud stage and the tentacled stage, as indicated by the shift from short multiple flashes to "coordinated" broad flashes in the KCl tests. In the latter stage, the presence of a conduction system common to the eight septa, and thus linking the photocyte groups, can be inferred.

The responses of the tentacle-bud stage juveniles are reminiscent of recordings obtained by Nicol (1955a) during bridge experiments on adult *Renilla*. With a small bridge of tissue separating two lobes of the rachis, several stimuli were required before luminous waves were observed in the distal lobe. Interneuronal facilitation (Pantin, 1935) within the colonial conduction system was proposed as a vehicle for the response. Similar experiments on two other pennatulids, *Stylatula* and *Virgularia*, using electrophysiological recordings of colonial nerve net activity, support this interpretation (Satterlie, Anderson and Case, in prepara-

tion). Immature juvenile conduction systems, or incomplete connections between systems could give rise to local responses. If so, the maturation of the conduction systems or connections, as well as that of the neuro-effector junctions could be represented by a gradual shift to a 1:1 stimulus-to-response ratio with a facilitating intensity output (Figs. 21C-F).

There are two colonial conduction systems in adult specimens of *Renilla*, in addition to semi-autonomous polyp conduction systems (Anderson and Case, 1975). We have demonstrated that at least one conduction system is present in the swimming planula prior to settlement. This conduction system mediates muscle activity of the entire larva (future polyp and colony tissue), and may represent the future colonial nerve net. At the time of settlement and metamorphosis the separation between the polyp and peduncular systems that becomes evident probably involves separation and elaboration of the polyp conduction system(s) and formation of colony-polyp connections. In light of the morphological reorganization that occurs in other pennatulids (*Ptilosarcus*, Chia and Crawford, 1977) at metamorphosis, reorganization of conduction systems in *Renilla* is certainly possible. The final stage in development of the neuro-effector systems, the appearance of facilitating responses, probably involves maturation of the neuro-effector junctions themselves. The role of the second colonial conduction systems of adult *Renilla* colonies is uncertain, and this report does not contribute to its elucidation.

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#### SUMMARY

1. The development of the pennatulid coelenterate *Renilla köllikeri* was followed from fertilized egg to primary polyp stage, including observations on the development of effector responses such as ciliary swimming, muscular reactions and bioluminescence.

2. Ciliary swimming is first apparent in the early planula, 32 hr after the spawn. Ciliary activity persists until settlement, at approximately 130 hr.

3. Muscular reactions are first evident as local contractions in the swimmer stage (about 60 hr). Conducted contractions can be elicited at 80 hr, suggesting that a functional conduction system is present in the planula larva. At settlement, separate peduncular and anthocodial muscular responses and peristaltic contractions are evident.

4. The future photocytes first fluoresce at about 80 hr, and thereafter fluorescence intensity increases until the time of settlement. The first sign of bioluminescence follows settlement, and is delayed if settlement is prevented. Bioluminescent responses do not exhibit normal facilitation until the primary polyp stage, and responses of the preceding stages may reflect maturation of the

colonial and polyp conduction systems as well as of connections between the two systems.

5. Settlement and metamorphosis are delayed when planulae are reared in containers without sand.

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